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=> "colorimetric fusion assay"
L1 0 "COLORIMETRIC FUSION ASSAY"

=> "fusion assay"
L2 788 "FUSION ASSAY"

=> HL2/3 and L2
'3' IS NOT A VALID FIELD CODE
'3' IS NOT A VALID FIELD CODE
L3 0 HL2/3 AND L2

=> HL2 and L2
L4 0 HL2 AND L2

=> Hela and l2
L5 44 HELA AND L2

=> transcriptional (w) factor
L6 3882 TRANSCRIPTIONAL (W) FACTOR

=> L6 and L5
L7 0 L6 AND L5

=> tat and L5
L8 6 TAT AND L5

=> LTR and L5
L9 10 LTR AND L5

=> D L9 IBIB ABS 1-10

L9 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:96746 CAPLUS
DOCUMENT NUMBER: 140:233560
TITLE: Expression cloning of functional receptor used by SARS coronavirus
AUTHOR(S): Wang, Peigang; Chen, Jian; Zheng, Aihua; Nie, Yuchun; Shi, Xuanling; Wang, Wei; Wang, Guangwen; Luo, Min; Liu, Huijun; Tan, Lei; Song, Xijun; Wang, Zai; Yin, Xiaolei; Qu, Xiuxia; Wang, Xiaojing; Qing, Tingting; Ding, Mingxiao; Deng, Hongkui
CORPORATE SOURCE: College of Life Sciences, Department of Cell Biology and Genetics, Peking University, Beijing, 100871, Peop. Rep. China
SOURCE: Biochemical and Biophysical Research Communications (2004), 315(2), 439-444
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have expressed a series of truncated spike (S) glycoproteins of SARS-CoV and found that the N-terminus 14-502 residuals were sufficient to bind to SARS-CoV susceptible Vero E6 cells. With this soluble S protein fragment as an affinity ligand, the authors screened **HeLa** cells transduced with retroviral cDNA library from Vero E6 cells and obtained a **HeLa** cell clone which could bind with the S protein. This cell

clone was susceptible to HIV/SARS pseudovirus infection and the presence of a functional receptor for S protein in this cell clone was confirmed by the cell-cell **fusion assay**. Further studies showed the susceptibility of this cell was due to the expression of endogenous angiotensin-converting enzyme 2 (ACE2) which was activated by inserted **LTR** from retroviral vector used for expression cloning. When human ACE2 cDNA was transduced into NIH3T3 cells, the ACE2 expressing NIH3T3 cells could be infected with HIV/SARS pseudovirus. These data clearly demonstrated that ACE2 was the functional receptor for SARS-CoV.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:800024 CAPLUS
DOCUMENT NUMBER: 132:160768
TITLE: The use of a quantitative **fusion assay** to evaluate HN-receptor interaction for human parainfluenza virus type 3
AUTHOR(S): Levin Perlman, Stephanie; Jordan, Maureen; Brossmer, Reinhard; Greengard, Olga; Moscona, Anne
CORPORATE SOURCE: Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, 10029-6574, USA
SOURCE: Virology (1999), 265(1), 57-65
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sialic acid is the receptor determinant for the human parainfluenza virus type 3 (HPF3) hemagglutinin-neuraminidase (HN) glycoprotein, the mol. responsible for binding of the virus to cell surfaces. In order for the fusion protein (F) of HPF3 to promote membrane fusion, HN must interact with its receptor. In addition to its role in receptor binding and fusion promotion, the HPF3 HN mol. contains receptor-destroying (sialidase) activity. The putative active sites are in the extracellular domain of this type II integral membrane protein. However, HN is not available in crystalline form; the exact locations of these sites, and the structural requirements for binding to the cellular receptor, which has not yet been isolated, are unknown. Nor have small mol. synthetic inhibitors of attachment or fusion that would provide insight into these processes been identified. The strategy in the present study was to develop an assay system that would provide a measure of a specific step in the viral cycle-functional interaction between viral glycoproteins and the cell during attachment and fusion-and serve to screen a variety of substances for inhibitory potential. The assay is based on our previous finding that CV-1 cells persistently infected (p.i.) with HPF3 do not fuse with one another but that the addition of uninfected CV-1 cells, supplying the critical sialic acid containing receptor mols. that bind HN, results in rapid fusion. In the present assay two **HeLa** cell types were used: we persistently infected **HeLa-LTR**-βgal cells, assessed their fusion with uninfected **HeLa**-tat cells, and then quantitated the β-galactosidase (βgal) produced as a result of this fusion. The analog α-2-S-methyl-5-N-thioacetylneuraminic acid (α-Neu5thioAc2SMe) interfered with fusion, decreasing βgal production by 84% at 50 mM and by 24% at 25 mM. In beginning to extend our studies to different types of mols., we tested an unsatd. derivative of sialic acid, 2,3-dehydro-2-deoxy-n-acetyl neuraminic acid (DANA), which is known to inhibit influenza neuraminidase by virtue of being a transition-state analog. We found that 10 mM DANA inhibited neuraminidase activity in HPF3 viral prepns. More significantly, this compound was active in our assay of HN-receptor interaction; 10 mM DANA completely blocked fusion and βgal production, and hemadsorption inhibition by DANA suggested that DANA blocks attachment. In plaque reduction assays performed with the compds., the active analog α-Neu5thioAc2SMe reduced plaque formation by 50% at a 50 mM concentration; DANA caused a 90% inhibition in the plaque reduction assay at a

concentration of 25 mM. Our results indicate that specific sialic acid analogs that mimic the cellular receptor determinant of HPF3 can block virus cell interaction and that an unsatd. n-acetyl-neuraminic acid derivative with affinity to the HN site responsible for neuraminidase activity also interferes with HN-receptor binding. Strategies suggested by these findings are now being pursued to obtain information regarding the relative locations of the active sites of HN and to further elucidate the relationship between the receptor-binding and receptor-destroying activities of HN during the viral life cycle. The quant. assay that we describe is of immediate applicability to large-scale screening for potential inhibitors of HPF3 infection in vivo. (c) 1999 Academic Press.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:204015 CAPLUS

DOCUMENT NUMBER: 129:80

TITLE: A simple assay system for examination of the inhibitory potential in vivo of decoy RNAs, ribozymes and other drugs by measuring the Tat-mediated transcription of a fusion gene composed of the long terminal repeat of HIV-1 and a gene for luciferase

AUTHOR(S): Koseki, Shiori; Ohkawa, Jun; Yamamoto, Rika; Takebe, Yutaka; Taira, Kazunari

CORPORATE SOURCE: MITI, National Institute of Bioscience and Human Technology, 1-1 Higashi, Tsukuba Science City, 305, Japan

SOURCE: Journal of Controlled Release (1998), 53(1-3), 159-173
CODEN: JCREEC; ISSN: 0168-3659

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Nucleic acid-based drugs, including antisense RNA and DNA, ribozymes and decoys appear to have potential for the suppression of the expression of specific genes. To allow the examination of the potential of such agents in vivo as anti-HIV drugs in standard labs., where facilities for handling live virions are not available, we constructed a simple assay system (HIV-1 model) that allows measurement of the extent of inhibition of Tat-mediated transcription of HIV-1 by nucleic acid-based drugs and other agents. In cells that harbor a stable chimeric long terminal repeat (**LTR**)-Luc construct (a fusion gene consisting of the **LTR** of HIV-1 and the gene for luciferase), total luciferase activity in an aliquot of cell lysate is dose- and promoter-dependent on transfection with a Tat expression plasmid, reflecting the character of the **LTR** promoter of HIV. When **HeLa** cells were co-transfected with the Tat expression plasmid and another plasmid that encoded the U6 promoter or the promoter of the gene for tRNAVal linked to the trans-activating response (TAR) sequence, total luciferase activity was inhibited by 60 or 40%, resp. The inhibition was also dependent on the dose of the TAR expression plasmid. These results demonstrate the usefulness of this simple assay system for detection of the efficacy of a decoy RNA or a ribozyme in vivo, without a requirement for HIV-infected cells, by measurement of luciferase activity in vitro.

REFERENCE COUNT: 72 THERE ARE 72 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:438785 CAPLUS

DOCUMENT NUMBER: 125:136768

TITLE: Expression of HIV env gene in a human T cell line for a rapid and quantifiable cell **fusion assay**

AUTHOR(S): Moir, Susan; Poulin, Louise

CORPORATE SOURCE: Faculty Medicine, Laval University, Ste-Foy, QC, G1V

4G2, Can.
SOURCE: AIDS Research and Human Retroviruses (1996), 12(9), 811-820
CODEN: ARHRE7; ISSN: 0889-2229
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins present at the surface of infected cells are known to mediate fusion with CD4-pos. target cells. In this study we have developed a novel Env-expressing cell line for investigating the fusion process in a biol. significant system. Cell surface expression of the HIV-1 env gene, isolated from the highly fusogenic strain SF33, was obtained in the CD4-neg. T cell line A2.01. To render the system versatile and efficient, HIV-1 regulatory proteins Tat and Rev were supplied in trans. The presence of Env at the cell surface was shown by cytofluorometry and immunofluorescence and precursor processing of gp160 to gp120/gp41 was demonstrated by Western blot. The fusion capacity of A2.01-Env cells was assessed by coculture with CD4-pos. T lymphocytes or the fusion indicator cell line, **HeLa**-CD4-**LTR**-β-Gal. By coincubation with CD4-pos. T cells such as SupT1, A2.01-Env cells were observed to mediate rapidly numerous well-defined syncytia in a reproducible fashion. By expressing Tat, they also had the capacity to trans-activate the **LTR**-linked reporter β-Gal gene following fusion with **HeLa**-CD4-**LTR**-β-Gal cells. The fusion-inhibiting anti-CD4 monoclonal antibodies Q425 and Q428 were used to block specifically Env-mediated fusion with CD4-pos. cells and to demonstrate application of this system to the search for potential fusion-blocking agents. Our system thus offers a biol. significant model for studying fusion events with the advantages of being rapid, reproducible, and versatile.

L9 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:19715 CAPLUS
DOCUMENT NUMBER: 124:105735
TITLE: Characterization of siamycin I, a human immunodeficiency virus fusion inhibitor
AUTHOR(S): Lin, Ping-Fang; Samanta, Himadri; Bechtold, Clifford M.; Deminie, Carol A.; Patick, Amy K.; Alam, Masud; Riccardi, Keith; Rose, Ronald E.; White, Richard J.; Colonno, Richard J.
CORPORATE SOURCE: Dep. Virol., Bristol-Myers Squibb Pharmaceutical Res. Inst., Wallingford, CT, 06492, USA
SOURCE: Antimicrobial Agents and Chemotherapy (1996), 40(1), 133-8
CODEN: AMACCQ; ISSN: 0066-4804
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The human immunodeficiency virus (HIV) fusion inhibitor siamycin I, a 21-residue tricyclic peptide, was identified from a Streptomyces culture by using a cell **fusion assay** involving cocultivation of **HeLa**-CD4+ cells and monkey kidney (BSC-1) cells expressing the HIV envelope gp160. Siamycin I is effective against acute HIV type 1 (HIV-1) and HIV-2 infections, with 50% EDs ranging from 0.05 to 5.7 μM, and the concentration resulting in a 50% decrease in cell viability in the absence of viral infection is 150 μM in CEM-SS cells. Siamycin I inhibits fusion between C8166 cells and CEM-SS cells chronically infected with HIV (50% ED of 0.08 μM) but has no effect on Sendai virus-induced fusion or murine myoblast fusion. Siamycin I does not inhibit gp120 binding to CD4 in either gp120- or CD4-based capture enzyme-linked immunosorbent assays. Inhibition of HIV-induced fusion by this compound is reversible, suggesting that siamycin I binds noncovalently. An HIV-1 resistant variant was selected by in vitro passage of virus in the presence of increasing concns. of siamycin I. Drug susceptibility studies

on a chimeric virus containing the envelope gene from the siamycin I-resistant variant indicate that resistance maps to the gp160 gene. Envelope-deficient HIV complemented with gp160 from siamycin I-resistant HIV also displayed a resistant phenotype upon infection of **HeLa** -CD4-**LTR**-β-gal cells. A comparison of the DNA sequences of the envelope genes from the resistant and parent viruses revealed a total of six amino acid changes. Together these results indicate that siamycin I interacts with the HIV envelope protein.

L9 ANSWER 6 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:158241 BIOSIS
DOCUMENT NUMBER: PREV200400158372
TITLE: Expression cloning of functional receptor used by SARS coronavirus.
AUTHOR(S): Wang, Peigang; Chen, Jian; Zheng, Aihua; Nie, Yuchun; Shi, Xuanling; Wang, Wei; Wang, Guangwen; Luo, Min; Liu, Huijun; Tan, Lei; Song, Xijun; Wang, Zai; Yin, Xiaolei; Qu, Xiuxia; Wang, Xiaojing; Qing, Tingting; Ding, Mingxiao [Reprint Author]; Deng, Hongkui [Reprint Author]
CORPORATE SOURCE: Department of Cell Biology and Genetics, College of Life Sciences, Peking University, Beijing, 100871, China hongkui_deng@pku.edu.cn
SOURCE: Biochemical and Biophysical Research Communications, (March 5 2004) Vol. 315, No. 2, pp. 439-444. print. CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Mar 2004 Last Updated on STN: 17 Mar 2004

AB We have expressed a series of truncated spike (S) glycoproteins of SARS-CoV and found that the N-terminus 14-502 residuals were sufficient to bind to SARS-CoV susceptible Vero E6 cells. With this soluble S protein fragment as an affinity ligand, we screened **HeLa** cells transduced with retroviral cDNA library from Vero E6 cells and obtained a **HeLa** cell clone which could bind with the S protein. This cell clone was susceptible to HIV/SARS pseudovirus infection and the presence of a functional receptor for S protein in this cell clone was confirmed by the cell-cell **fusion assay**. Further studies showed the susceptibility of this cell was due to the expression of endogenous angiotensin-converting enzyme 2 (ACE2) which was activated by inserted **LTR** from retroviral vector used for expression cloning. When human ACE2 cDNA was transduced into NIH3T3 cells, the ACE2 expressing NIH3T3 cells could be infected with HIV/SARS pseudovirus. These data clearly demonstrated that ACE2 was the functional receptor for SARS-CoV.

L9 ANSWER 7 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:218273 BIOSIS
DOCUMENT NUMBER: PREV200000218273
TITLE: Human T-cell leukemia virus type 1 Tax shuttles between functionally discrete subcellular targets.
AUTHOR(S): Burton, Molly; Upadhyaya, Cherrag D.; Maier, Bernhard; Hope, Thomas J.; Semmes, O. John [Reprint author]
CORPORATE SOURCE: Department of Microbiology, University of Virginia School of Medicine, Jordan Hall 7-89, Charlottesville, VA, 23060, USA
SOURCE: Journal of Virology, (March, 2000) Vol. 74, No. 5, pp. 2351-2364. print. CODEN: JOVIAM. ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 May 2000 Last Updated on STN: 5 Jan 2002

AB Human T-cell leukemia virus type 1 (HTLV-1) Tax is a nuclear protein with striking pleiotropic functionality. We recently demonstrated that Tax localizes to a multicomponent nuclear speckled structure (Tax speckled structure (TSS)). Here, we examine these structures further and identify a partial overlap of TSS with transcription hot spots. We used a strategy of directed expression via fusion proteins to determine if these transcription sites are the subtargets within TSS required for Tax function. When fused to human immunodeficiency virus type 1 (HIV-1) Tat, the resulting Tat-Tax fusion protein displayed neither a Tat-like nor a Tax-like pattern but rather was targeted specifically to the transcription subsites. The Tat-Tax fusion was able to activate both the HIV-1 long terminal repeat (**LTR**) and the HTVL-1 **LTR** at the same level as the individual component; thus, targeting proteins to transcription hot spots was compatible with both Tax and Tat transcription function. In contrast, the fusion with HIV-1 Rev, Rev-Tax, resulted in a pattern of expression that was largely Rev-like (nucleolar and cytoplasmic). The reduced localization of Rev-Tax to transcription sites was reflected in a 10-fold drop in activation of the HTLV-1 **LTR**. However, there was no loss in the ability of Tax to activate via NF-kappaB. Thus, NF-kappaB-dependent Tax function does not require targeting of Tax to these transcription sites and suggests that activation via NF-kappaB is a cytoplasmic function. Selective mutation of the nuclear localization signal site in the Rev portion resulted in retargeting of Rev-Tax to TSS and subsequent restoration of transcription function, demonstrating that inappropriate localization preceded loss of function. Mutation of the nuclear export signal site in the Rev portion had no effect on transcription, although the relative amount of Rev-Tax in the cytoplasm was reduced. Finally, in explaining how Tax can occupy multiple subcellular sites, we show that Tax shuttles from the nucleus to the cytoplasm in a heterokaryon **fusion assay**. Thus, pleiotropic functionality by Tax is regulatable via shuttling between discrete subcellular compartments.

L9 ANSWER 8 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:77163 BIOSIS
DOCUMENT NUMBER: PREV200000077163
TITLE: The use of a quantitative **fusion assay** to evaluate HN-receptor interaction for human parainfluenza virus type 3.
AUTHOR(S): Perlman, Stephanie Levin; Jordan, Maureen; Brossmer, Reinhard; Greengard, Olga; Moscona, Anne [Reprint author]
CORPORATE SOURCE: Department of Pediatrics, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY, USA
SOURCE: Virology, (Dec. 5, 1999) Vol. 265, No. 1, pp. 57-65. print. CODEN: VIRLAX. ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Feb 2000
Last Updated on STN: 3 Jan 2002

AB Sialic acid is the receptor determinant for the human parainfluenza virus type 3 (HPF3) hemagglutinin-neuraminidase (HN) glycoprotein, the molecule responsible for binding of the virus to cell surfaces. In order for the fusion protein (F) of HPF3 to promote membrane fusion, HN must interact with its receptor. In addition to its role in receptor binding and fusion promotion, the HPF3 HN molecule contains receptor-destroying (sialidase) activity. The putative active sites are in the extracellular domain of this type II integral membrane protein. However, HN is not available in crystalline form; the exact locations of these sites, and the structural requirements for binding to the cellular receptor, which has not yet been isolated, are unknown. Nor have small molecular synthetic inhibitors of attachment or fusion that would provide insight into these processes been identified. The strategy in the present study was to develop an assay system that would provide a measure of a specific step in the viral

cycle-functional interaction between viral glycoproteins and the cell during attachment and fusion-and serve to screen a variety of substances for inhibitory potential. The assay is based on our previous finding that CV-1 cells persistently infected (p.i.) with HPF3 do not fuse with one another but that the addition of uninfected CV-1 cells, supplying the critical sialic acid containing receptor molecules that bind HN, results in rapid fusion. In the present assay two **HeLa** cell types were used: we persistently infected **HeLa-LTR**-betagal cells, assessed their fusion with uninfected **HeLa**-tat cells, and then quantitated the beta-galactosidase (betagal) produced as a result of this fusion. The analog alpha-2-S-methyl-5-N-thioacetylneuraminic acid (alpha-Neu5thioAc2SMe) interfered with fusion, decreasing betagal production by 84% at 50 mM and by 24% at 25 mM. In beginning to extend our studies to different types of molecules, we tested an unsaturated derivative of sialic acid, 2,3-dehydro-2-deoxy-n-acetyl neuraminic acid (DANA), which is known to inhibit influenza neuraminidase by virtue of being a transition-state analog. We found that 10 mM DANA inhibited neuraminidase activity in HPF3 viral preparations. More significantly, this compound was active in our assay of HN-receptor interaction; 10 mM DANA completely blocked fusion and betagal production, and hemadsorption inhibition by DANA suggested that DANA blocks attachment. In plaque reduction assays performed with the compounds, the active analog alpha-Neu5thioAc2SMe reduced plaque formation by 50% at a 50 mM concentration; DANA caused a 90% inhibition in the plaque reduction assay at a concentration of 25 mM. Our results indicate that specific sialic acid analogs that mimic the cellular receptor determinant of HPF3 can block virus cell interaction and that an unsaturated n-acetyl-neuraminic acid derivative with affinity to the HN site responsible for neuraminidase activity also interferes with HN-receptor binding. Strategies suggested by these findings are now being pursued to obtain information regarding the relative locations of the active sites of HN and to further elucidate the relationship between the receptor-binding and receptor-destroying activities of HN during the viral life cycle. The quantitative assay that we describe is of immediate applicability to large-scale screening for potential inhibitors of HPF3 infection in vivo.

L9 ANSWER 9 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1997:42494 BIOSIS
DOCUMENT NUMBER: PREV199799334482

TITLE: Expression of HIV env gene in a human T cell line for a rapid and quantifiable cell **fusion assay**

AUTHOR(S): Moir, Susan; Poulin, Louise [Reprint author]
CORPORATE SOURCE: Infectiol., Cent. Recherche du CHUL, 2705 Boul. Laurier, Ste-Foy, Quebec G1V 4G2, Canada

SOURCE: AIDS Research and Human Retroviruses, (1996) Vol. 12, No. 9, pp. 811-820.
CODEN: ARHRE7. ISSN: 0889-2229.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 28 Jan 1997

AB Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins present at the surface of infected cells are known to mediate fusion with CD4-positive target cells. In this study we have developed a novel Env-expressing cell line for investigating the fusion process in a biologically significant system. Cell surface expression of the HIV-1 env gene, isolated from the highly fusogenic strain SF33, was obtained in the CD4-negative T cell line A2.01. To render the system versatile and efficient, BIV-1 regulatory proteins Tat and Rev were supplied in trans. The presence of Env at the cell surface was shown by cytofluorometry and immunofluorescence and precursor processing of gp160 to gp120/gp41 was demonstrated by Western blot. The fusion capacity of A2.01-Env cells was

assessed by coculture with CD4-positive T lymphocytes or the fusion indicator cell line, **HeLa**-CD4-**LTR**-beta-Gal. By coincubation with CD4-positive T cells such as SupT1, A2.01-Env cells were observed to mediate rapidly numerous well-defined syncytia in a reproducible fashion. By expressing Tat, they also had the capacity to trans-activate the **LTR**-linked reporter beta-Gal gene following fusion with **HeLa**-CD4-**LTR**-beta-Gal cells. The fusion-inhibiting anti-CD4 monoclonal antibodies Q425 and Q428 were used to block specifically Env-mediated fusion with CD4-positive cells and to demonstrate application of this system to the search for potential fusion-blocking agents. Our system thus offers a biologically significant model for studying fusion events with the advantages of being rapid, reproducible, and versatile.

L9 ANSWER 10 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1996:73061 BIOSIS
DOCUMENT NUMBER: PREV199698645196
TITLE: Characterization of siamycin I, a human immunodeficiency virus fusion inhibitor.
AUTHOR(S): Lin, Pin-Fang [Reprint author]; Samanta, Himadri; Bechtold, Clifford M.; Deminie, Carol A.; Patick, Amy K.; Alam, Masud; Riccardi, Keith; Rose, Ronald E.; White, Richard J.; Colonno, Richard J.
CORPORATE SOURCE: Bristol-Myeres Squibb Co., 5 Research Parkway, Wallingford, CT 06492, USA
SOURCE: Antimicrobial Agents and Chemotherapy, (1996) Vol. 40, No. 1, pp. 133-138.
CODEN: AMACCQ. ISSN: 0066-4804.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Feb 1996
Last Updated on STN: 27 Feb 1996

AB The human immunodeficiency virus (HIV) fusion inhibitor siamycin I, a 21-residue tricyclic peptide, was identified from a Streptomyces culture by using a cell **fusion assay** involving cocultivation of **HeLa**-CD4+ cells and monkey kidney (BSC-1) cells expressing the HIV envelope gp160. Siamycin I is effective against acute HIV type 1 (HIV-1) and HIV-2 infections, with 50% effective doses ranging from 0.05 to 5.7 mu-M, and the concentration resulting in a 50% decrease in cell viability in the absence of viral infection is 150 mu-M in CEM-SS cells. Siamycin I inhibits fusion between C8166 cells and CEM-SS cells chronically infected with HIV (50% effective dose of 0.08 mu-M) but has no effect on Sendai virus-induced fusion or murine myoblast fusion. Siamycin I does not inhibit gp120 binding to CD4 in either gp120- or CD4-based capture enzyme-linked immunosorbent assays. Inhibition of HIV-induced fusion by this compound is reversible, suggesting that siamycin I binds noncovalently. An HIV-1 resistant variant was selected by in vitro passage of virus in the presence of increasing concentrations of siamycin I. Drug susceptibility studies on a chimeric virus containing the envelope gene from the siamycin I-resistant variant indicate that resistance maps to the gp160 gene. Envelope-deficient HIV complemented with gp160 from siamycin I-resistant HIV also displayed a resistant phenotype upon infection of **HeLa**-CD4-**LTR**-beta-gal cells. A comparison of the DNA sequences of the envelope genes from the resistant and parent viruses revealed a total of six amino acid changes. Together these results indicate that siamycin I interacts with the HIV envelope protein.

=> D L8 IBIB ABS 1-6

L8 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:800024 CAPLUS

DOCUMENT NUMBER: 132:160768
TITLE: The use of a quantitative **fusion assay** to evaluate HN-receptor interaction for human parainfluenza virus type 3
AUTHOR(S): Levin Perlman, Stephanie; Jordan, Maureen; Brossmer, Reinhard; Greengard, Olga; Moscona, Anne
CORPORATE SOURCE: Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, 10029-6574, USA
SOURCE: Virology (1999), 265(1), 57-65
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sialic acid is the receptor determinant for the human parainfluenza virus type 3 (HPF3) hemagglutinin-neuraminidase (HN) glycoprotein, the mol. responsible for binding of the virus to cell surfaces. In order for the fusion protein (F) of HPF3 to promote membrane fusion, HN must interact with its receptor. In addition to its role in receptor binding and fusion promotion, the HPF3 HN mol. contains receptor-destroying (sialidase) activity. The putative active sites are in the extracellular domain of this type II integral membrane protein. However, HN is not available in crystalline form; the exact locations of these sites, and the structural requirements for binding to the cellular receptor, which has not yet been isolated, are unknown. Nor have small mol. synthetic inhibitors of attachment or fusion that would provide insight into these processes been identified. The strategy in the present study was to develop an assay system that would provide a measure of a specific step in the viral cycle-functional interaction between viral glycoproteins and the cell during attachment and fusion-and serve to screen a variety of substances for inhibitory potential. The assay is based on our previous finding that CV-1 cells persistently infected (p.i.) with HPF3 do not fuse with one another but that the addition of uninfected CV-1 cells, supplying the critical sialic acid containing receptor mols. that bind HN, results in rapid fusion. In the present assay two **HeLa** cell types were used: we persistently infected **HeLa**-LTR-βgal cells, assessed their fusion with uninfected **HeLa-tat** cells, and then quantitated the β-galactosidase (βgal) produced as a result of this fusion. The analog α-2-S-methyl-5-N-thioacetylneuraminic acid (α-Neu5thioAc2SMe) interfered with fusion, decreasing βgal production by 84% at 50 mM and by 24% at 25 mM. In beginning to extend our studies to different types of mols., we tested an unsatd. derivative of sialic acid, 2,3-dehydro-2-deoxy-n-acetyl neuraminic acid (DANA), which is known to inhibit influenza neuraminidase by virtue of being a transition-state analog. We found that 10 mM DANA inhibited neuraminidase activity in HPF3 viral prepns. More significantly, this compound was active in our assay of HN-receptor interaction; 10 mM DANA completely blocked fusion and βgal production, and hemadsorption inhibition by DANA suggested that DANA blocks attachment. In plaque reduction assays performed with the compds., the active analog α-Neu5thioAc2SMe reduced plaque formation by 50% at a 50 mM concentration; DANA caused a 90% inhibition in the plaque reduction assay at a concentration of 25 mM. Our results indicate that specific sialic acid analogs that mimic the cellular receptor determinant of HPF3 can block virus cell interaction and that an unsatd. n-acetyl-neuraminic acid derivative with affinity to the HN site responsible for neuraminidase activity also interferes with HN-receptor binding. Strategies suggested by these findings are now being pursued to obtain information regarding the relative locations of the active sites of HN and to further elucidate the relationship between the receptor-binding and receptor-destroying activities of HN during the viral life cycle. The quant. assay that we describe is of immediate applicability to large-scale screening for potential inhibitors of HPF3 infection in vivo. (c) 1999 Academic Press.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:204015 CAPLUS
DOCUMENT NUMBER: 129:80
TITLE: A simple assay system for examination of the inhibitory potential in vivo of decoy RNAs, ribozymes and other drugs by measuring the **Tat** -mediated transcription of a fusion gene composed of the long terminal repeat of HIV-1 and a gene for luciferase
AUTHOR(S): Koseki, Shiori; Ohkawa, Jun; Yamamoto, Rika; Takebe, Yutaka; Taira, Kazunari
CORPORATE SOURCE: MITI, National Institute of Bioscience and Human Technology, 1-1 Higashi, Tsukuba Science City, 305, Japan
SOURCE: Journal of Controlled Release (1998), 53(1-3), 159-173
CODEN: JCREEC; ISSN: 0168-3659
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Nucleic acid-based drugs, including antisense RNA and DNA, ribozymes and decoys appear to have potential for the suppression of the expression of specific genes. To allow the examination of the potential of such agents in vivo as anti-HIV drugs in standard labs., where facilities for handling live virions are not available, we constructed a simple assay system (HIV-1 model) that allows measurement of the extent of inhibition of **Tat** -mediated transcription of HIV-1 by nucleic acid-based drugs and other agents. In cells that harbor a stable chimeric long terminal repeat (LTR)-Luc construct (a fusion gene consisting of the LTR of HIV-1 and the gene for luciferase), total luciferase activity in an aliquot of cell lysate is dose- and promoter-dependent on transfection with a **Tat** expression plasmid, reflecting the character of the LTR promoter of HIV. When **HeLa** cells were co-transfected with the **Tat** expression plasmid and another plasmid that encoded the U6 promoter or the promoter of the gene for tRNAVal linked to the trans-activating response (TAR) sequence, total luciferase activity was inhibited by 60 or 40%, resp. The inhibition was also dependent on the dose of the TAR expression plasmid. These results demonstrate the usefulness of this simple assay system for detection of the efficacy of a decoy RNA or a ribozyme in vivo, without a requirement for HIV-infected cells, by measurement of luciferase activity in vitro.
REFERENCE COUNT: 72 THERE ARE 72 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:438785 CAPLUS
DOCUMENT NUMBER: 125:136768
TITLE: Expression of HIV env gene in a human T cell line for a rapid and quantifiable cell **fusion assay**
AUTHOR(S): Moir, Susan; Poulin, Louise
CORPORATE SOURCE: Faculty Medicine, Laval University, Ste-Foy, QC, G1V 4G2, Can.
SOURCE: AIDS Research and Human Retroviruses (1996), 12(9), 811-820
CODEN: ARHRE7; ISSN: 0889-2229
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins present at the surface of infected cells are known to mediate fusion with CD4-pos. target cells. In this study we have developed a novel Env-expressing cell line for investigating the fusion process in a biol. significant system. Cell surface expression of the HIV-1 env gene, isolated from the highly

fusogenic strain SF33, was obtained in the CD4-neg. T cell line A2.01. To render the system versatile and efficient, HIV-1 regulatory proteins **Tat** and Rev were supplied in trans. The presence of Env at the cell surface was shown by cytofluorometry and immunofluorescence and precursor processing of gp160 to gp120/gp41 was demonstrated by Western blot. The fusion capacity of A2.01-Env cells was assessed by coculture with CD4-pos. T lymphocytes or the fusion indicator cell line, **HeLa**-CD4-LTR-β-Gal. By coincubation with CD4-pos. T cells such as SupT1, A2.01-Env cells were observed to mediate rapidly numerous well-defined syncytia in a reproducible fashion. By expressing **Tat**, they also had the capacity to trans-activate the LTR-linked reporter β-Gal gene following fusion with **HeLa** -CD4-LTR-β-Gal cells. The fusion-inhibiting anti-CD4 monoclonal antibodies Q425 and Q428 were used to block specifically Env-mediated fusion with CD4-pos. cells and to demonstrate application of this system to the search for potential fusion-blocking agents. Our system thus offers a biol. significant model for studying fusion events with the advantages of being rapid, reproducible, and versatile.

L8 ANSWER 4 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:218273 BIOSIS
DOCUMENT NUMBER: PREV200000218273
TITLE: Human T-cell leukemia virus type 1 Tax shuttles between functionally discrete subcellular targets.
AUTHOR(S): Burton, Molly; Upadhyaya, Cherrag D.; Maier, Bernhard; Hope, Thomas J.; Semmes, O. John [Reprint author]
CORPORATE SOURCE: Department of Microbiology, University of Virginia School of Medicine, Jordan Hall 7-89, Charlottesville, VA, 23060, USA
SOURCE: Journal of Virology, (March, 2000) Vol. 74, No. 5, pp. 2351-2364. print.
CODEN: JOVIAM. ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 May 2000
Last Updated on STN: 5 Jan 2002

AB Human T-cell leukemia virus type 1 (HTLV-1) Tax is a nuclear protein with striking pleiotropic functionality. We recently demonstrated that Tax localizes to a multicomponent nuclear speckled structure (Tax speckled structure (TSS)). Here, we examine these structures further and identify a partial overlap of TSS with transcription hot spots. We used a strategy of directed expression via fusion proteins to determine if these transcription sites are the subtargets within TSS required for Tax function. When fused to human immunodeficiency virus type 1 (HIV-1) **Tat**, the resulting **Tat**-Tax fusion protein displayed neither a **Tat**-like nor a Tax-like pattern but rather was targeted specifically to the transcription subsites. The **Tat** -Tax fusion was able to activate both the HIV-1 long terminal repeat (LTR) and the HTVL-1 LTR at the same level as the individual component; thus, targeting proteins to transcription hot spots was compatible with both Tax and **Tat** transcription function. In contrast, the fusion with HIV-1 Rev, Rev-Tax, resulted in a pattern of expression that was largely Rev-like (nucleolar and cytoplasmic). The reduced localization of Rev-Tax to transcription sites was reflected in a 10-fold drop in activation of the HTLV-1 LTR. However, there was no loss in the ability of Tax to activate via NF-kappaB. Thus, NF-kappaB-dependent Tax function does not require targeting of Tax to these transcription sites and suggests that activation via NF-kappaB is a cytoplasmic function. Selective mutation of the nuclear localization signal site in the Rev portion resulted in retargeting of Rev-Tax to TSS and subsequent restoration of transcription function, demonstrating that inappropriate localization preceded loss of function. Mutation of the nuclear export signal site in the Rev portion had no effect on transcription, although the relative amount of Rev-Tax in the cytoplasm was reduced. Finally, in explaining how Tax can occupy

multiple subcellular sites, we show that Tax shuttles from the nucleus to the cytoplasm in a heterokaryon **fusion assay**. Thus, pleiotropic functionality by Tax is regulatable via shuttling between discrete subcellular compartments.

L8 ANSWER 5 OF 6 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:77163 BIOSIS
DOCUMENT NUMBER: PREV200000077163
TITLE: The use of a quantitative **fusion assay** to evaluate HN-receptor interaction for human parainfluenza virus type 3.
AUTHOR(S): Perlman, Stephanie Levin; Jordan, Maureen; Brossmer, Reinhard; Greengard, Olga; Moscona, Anne [Reprint author]
CORPORATE SOURCE: Department of Pediatrics, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY, USA
SOURCE: Virology, (Dec. 5, 1999) Vol. 265, No. 1, pp. 57-65. print. CODEN: VIRLAX. ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Feb 2000
Last Updated on STN: 3 Jan 2002

AB Sialic acid is the receptor determinant for the human parainfluenza virus type 3 (HPF3) hemagglutinin-neuraminidase (HN) glycoprotein, the molecule responsible for binding of the virus to cell surfaces. In order for the fusion protein (F) of HPF3 to promote membrane fusion, HN must interact with its receptor. In addition to its role in receptor binding and fusion promotion, the HPF3 HN molecule contains receptor-destroying (sialidase) activity. The putative active sites are in the extracellular domain of this type II integral membrane protein. However, HN is not available in crystalline form; the exact locations of these sites, and the structural requirements for binding to the cellular receptor, which has not yet been isolated, are unknown. Nor have small molecular synthetic inhibitors of attachment or fusion that would provide insight into these processes been identified. The strategy in the present study was to develop an assay system that would provide a measure of a specific step in the viral cycle-functional interaction between viral glycoproteins and the cell during attachment and fusion-and serve to screen a variety of substances for inhibitory potential. The assay is based on our previous finding that CV-1 cells persistently infected (p.i.) with HPF3 do not fuse with one another but that the addition of uninfected CV-1 cells, supplying the critical sialic acid containing receptor molecules that bind HN, results in rapid fusion. In the present assay two **HeLa** cell types were used: we persistently infected **HeLa**-LTR-betagal cells, assessed their fusion with uninfected **HeLa-tat** cells, and then quantitated the beta-galactosidase (betagal) produced as a result of this fusion. The analog alpha-2-S-methyl-5-N-thioacetylneuraminic acid (alpha-Neu5thioAc2SMe) interfered with fusion, decreasing betagal production by 84% at 50 mM and by 24% at 25 mM. In beginning to extend our studies to different types of molecules, we tested an unsaturated derivative of sialic acid, 2,3-dehydro-2-deoxy-n-acetyl neuraminic acid (DANA), which is known to inhibit influenza neuraminidase by virtue of being a transition-state analog. We found that 10 mM DANA inhibited neuraminidase activity in HPF3 viral preparations. More significantly, this compound was active in our assay of HN-receptor interaction; 10 mM DANA completely blocked fusion and betagal production, and hemadsorption inhibition by DANA suggested that DANA blocks attachment. In plaque reduction assays performed with the compounds, the active analog alpha-Neu5thioAc2SMe reduced plaque formation by 50% at a 50 mM concentration; DANA caused a 90% inhibition in the plaque reduction assay at a concentration of 25 mM. Our results indicate that specific sialic acid analogs that mimic the cellular receptor determinant of HPF3 can block virus cell interaction and that an unsaturated n-acetyl-neuraminic acid derivative with affinity to the HN site responsible for neuraminidase activity also interferes with HN-receptor binding. Strategies suggested